

Brownian Walks in Conformational Space

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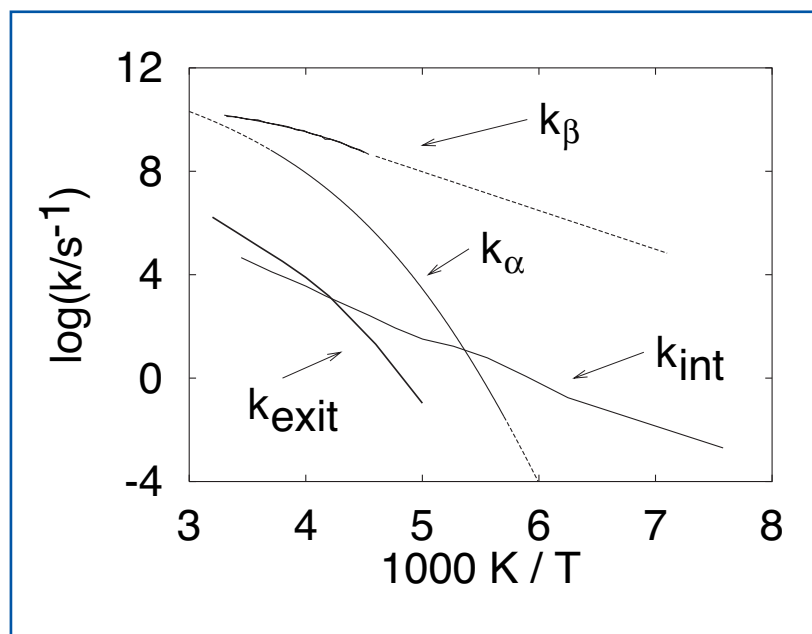
Brownian motion was the subject of one of the three fundamental papers that Einstein wrote in 1905. One hundred years later, it has become clear that Brownian motion, in real and in conformational space, is crucial for biological processes. Most biological processes are so complex that it is difficult to arrive at a description and understanding that is accessible to physicists and biologists. Some processes in proteins, the building blocks of biological systems, are however simple enough that a bridge between disciplines can be built. We discuss here one specific example, the role of fluctuations and relaxations in the dynamics and function of myoglobin (Mb), a protein whose main role is the storage of dioxygen. Myoglobin consists of 153 amino acids that fold into a globule. The globule incorporates a heme group with an iron atom in its center. The protein is clothed in the hydration shell, a layer of water molecules; the whole is embedded in a solvent. For storage, the O_2 enters Mb and

binds at the heme iron. X-ray structures, however, show no permanent channel through which O_2 or other small molecules such as CO could enter and leave Mb. Opening and closing must consequently be dynamic protein processes, caused by thermal fluctuations. Studying the transport process in detail as a function of temperature and time leads to some unexpected concepts that may be crucial for many biological protein processes and that also connect physics and biology intimately.

Figure 1 shows the temperature dependence of rate coefficients for processes in Mb: k_{exit} refers to the exit of a small ligand (CO) into the solvent after a laser flash has broken the bond between the iron and the CO molecule. k_{int} is the rate coefficient for the internal motion of CO inside the protein, and $k_{\alpha}(T)$ and $k_{\beta}(T)$ denote the rate coefficients for the dielectric relaxation in the solvent surrounding the protein and in the hydration shell, respectively. A number of features stand out in Fig. 1. The rate coefficient for the exit of CO follows the dielectric fluctuations in the solvent over a broad range, but it is slower by about a factor 10^5 . The rate for the CO motion inside the protein follows the dielectric fluctuations in the hydration shell but is slower by about a factor 10^6 . Since the temperature dependences of the protein processes, exit and internal motion, are given by the solvent and the hydration shell, the slowing cannot be due to enthalpy barriers in the protein. Slowing must be due to protein entropy barriers! How can this conclusion be understood?

Insight into the entropic barriers comes from the realization that a protein does not exist in a unique structure, as suggested by pictures shown in textbooks, but can assume a very large number of somewhat different conformations, called conformational substates (CS). A protein consequently must be described by an energy (or more correctly entropy) landscape (EL). The EL is a construct in $\approx 3N$ dimensions, where N denotes the number of atoms in the protein and its hydration shell. A point in the EL describes a particular conformation. The EL is organized hierarchically into a number of tiers. Figure 2 gives a 2D cross section through a tier that we call 1α ; cc1 and cc2 are

Figure 1—
The rate coefficient $k_{\text{exit}}(T)$ for the exit of CO from myoglobin has the same temperature dependence as the rate coefficient $k_{\alpha}(T)$ for dielectric fluctuations in the solvent surrounding Mb, but is much slower. Similarly, $k_{\text{int}}(T)$ for the internal motion of CO inside Mb follows $k_{\beta}(T)$, the fast fluctuations in the hydration shell.



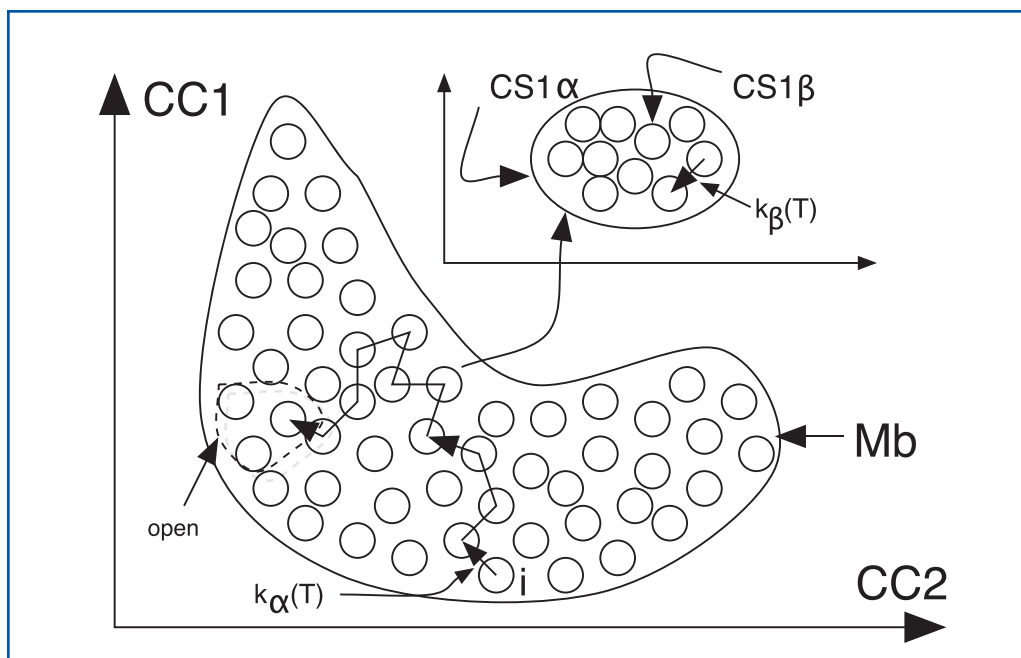


Figure 2—
A 2D cross section through the energy landscape of a typical protein, here Mb. *cc1* and *cc2* are two conformational coordinates. The main figure shows schematically the tier 1 α with a large number of *CS1 α* , shown as small circles. Within each *CS1 α* there are a large number of *CS1 β* . Transitions between *CS1 α* are assumed to be given by $k_{\alpha}(T)$, the fluctuation rate in the solvent. Transitions between *CS1 β* are given by the β -fluctuations in the hydration shell.

two of the 3N conformational coordinates. The small circles, denoted by *CS1 α* , represent substates. Experiments show that each *CS1 α* contains a large number of substates separated by smaller barriers. These are denoted by *CS1 β* . The EL in one particular *CS1 α* is sketched as an insert in Fig. 2.

The EL provides an intuitive explanation of why $k_{\text{exit}}(T)$ is much smaller than $k_{\alpha}(T)$, but follows $k_{\alpha}(T)$. Assume that each transition from one *CS1 α* to another one is given by $k_{\alpha}(T)$. Most *CS1 α* correspond to conformations with no open channel to the outside. A small number, denoted “open” in Fig. 2, correspond to open channels. A protein starting in some initial CS, denoted by *i*, makes a random walk in the conformational space until it comes to the open CS where the ligand can escape. The solvents thus slaves large-scale motions in the protein: the transition from one *CS1 α* to another one can take place only if the solvent fluctuates, and on average a large number of steps are needed before a channel opens. A similar argument explains why CO can only transit within the protein if the hydration shell fluctuates and why the $k_{\text{int}}(T)$ is so much smaller than $k_{\beta}(T)$. The large number of states in the hydration shell, i.e., its entropy, is crucial for the slow transit; in a dehydrated

protein, the CO cannot move through the protein or into the solvent.

The study of fluctuations in proteins is not only interesting for physics; it also provides lessons for the control of biological reactions in cells. If fluctuations in the solvent slave large-scale motions in proteins and if the hydration shell can control internal motions, the environment in cells has at least two ways to influence protein functions. Proteins are not isolated systems; they are regulated by the environment.

- [1] Albert Einstein, *Investigations on the Theory of the Brownian Movement* (Dover Publications, 1985).
- [2] H. Frauenfelder, S.G. Sligar, and P.G. Wolynes, *Science* **254**, 1598 (1991).
- [3] P.W. Fenimore, H. Frauenfelder, B.H. McMahon, and F.G. Parak, *Proc Natl. Acad. Sci. USA* **99**, 16047 (2002).
- [4] P.W. Fenimore, H. Frauenfelder, B.H. McMahon, and R.D. Young, *Proc. Natl. Acad. Sci. USA* **101**, 14408 (2004).
- [5] V. Lubchenko, P.G. Wolynes, and H. Frauenfelder, to be published in *J. Phys. Chem.*

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